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08/796, 164 and 08/874, 992

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Comments:

Here is a list of claims allowed in 08/667,003 (Issue Fee received at USPTO 18 October 2000) for you to consider in the related '164 and '992 applications. Carol

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Drell: 809 16

Allowed Claims in 08/667,003

::ODMA\MHODMA\IMANAGE;145560;1 Issue Fee received at Docker No.: 1818.1010-001
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07 July 7, 2000
USPTO 18 October 2000

CLAIMS AS AMENDED IN AMENDMENT AFTER FINAL REJECTION ON JULY

- (Twice Amended) A method for forming a composition comprising polynitrosated methemoglobin, comprising combining methemoglobin with an excess of S-nitrosothio over methemoglobin in an aqueous solution at a pH of about 7.4 to about 9.2, and maintaining the resulting combination under conditions appropriate for nitrosation to 35. maintaining the resulting combination under conditions appropriate for nitrosation to occur at multiple sites on methemoglobin, thereby forming a composition comprising polynitrosated methemoglobin.
- **36.** (Twice Amended) A method for forming a composition comprising polynitrosated or polynitrated oxyhemoglobin in which home Fe is in the FeII state, comprising combining oxyhemoglobin with an excess of nitrosothiol at a pH of about 7.4 to about 9.2, maintaining the resulting combination under conditions appropriate for nitrosation or nitration to occur, thereby forming a composition comprising polynitrosated or polynitrated methemoglobin, and reacting the polynitrosated or polynitrated methemoglobin with a reagent which selectively reduces FeIII to FeII, thereby forming a composition comprising polynitrosated or polynitrated oxyhemoglobin.
- 37. (Amended) The method of Claim 36 in which the reagent which selectively reduces FeIII to Pell is a cyanoborohydride.
- The method of Claim 36 in which the reagent which selectively reduces FeIII to FeII is 38. methemoglobin reductase.

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- A method for producing a composition comprising polynitrosated methomoglobin, comprising incubating oxyhemoglobin with about 100-fold excess S-nitrosoglutathione over protein at about pH 9.2 at about 25°C.
- 52. A method for producing a composition comprising SNO-methomoglobin having 2 SNO groups per hemoglobin tetranicr, comprising incubating oxylemoglobin with about 100-fold excess S-nitrosocysteine over hemoglobin tetramer, at about pH 7.4 at about 25°C.
- A method for preparing a composition comprising polynitrosated oxylemoglobin, said method comprising combining oxylemoglobin with an excess of S-nitrosocysteine, S-nitrosoglutathione, S-nitrosohomocysteine or S-nitrosocysteinylglycine over hemoglobin in an aqueous solution at a pH of about 7.4 to about 9.2, and maintaining the resulting combination under conditions appropriate for nitrosation to occur at multiple sites on hemoglobin, thereby producing polynitrosated hemoglobin.
- 55. A method for preparing a composition comprising polynitrosated or polynitrated hemoglobin in which hemo Fe is in the FeII state, said method comprising combining hemoglobin with S-nitrosocysteine, S-nitrosoglutathione, S-nitrosohomocysteine or S-nitrosocysteinylglycine at a pH of about 7.4 to about 9.2, maintaining the resulting combination under conditions appropriate for nitrosation or nitration to occur, thereby producing a composition comprising polynitrosated or polynitrated hemoglobin, and polynitrosated or polynitrated methemoglobin, and reacting the polynitrosated or polynitrated methemoglobin with a reagent which selectively reduces FeIII to FeII.

A method for preparing a composition comprising polynitrosated methemoglobin, comprising combining oxyhemoglobin with an excess of S-nitrosocysteine, S-nitrosoglutathione, S-nitrosohomocysteine or S-nitrosocysteinylglycine over methemoglobin in an aqueous solution at a pH of about 7.4 to about 9.2, and maintaining the resulting combination under conditions appropriate for nitrosation to occur at multiple sites on methemoglobin, thereby forming a composition comprising polynitrosated methemoglobin.